PART IV

Recent Advances

1946–47

The foregoing pages were completed during the summer of 1946. The galleys arrived as progress continued into 1947; and while at first the author considered incorporating into the original text the advances achieved, it seemed preferable for technical and other reasons simply to add a supplement. A section on methods has also been added.

I. Molecular Weight

O. Snellman and T. Erdös are engaged in measuring the MW of crystalline myosin in the ultracentrifuge. The first results suggest that crystalline myosin is homogeneous, and its MW is of the order of 10^6, in agreement with earlier results of Weber and Stöver.

II. Actin Content and Activity of Myosin

Actin and myosin, if brought together, unite to form the highly viscous actomyosin, which if dissolved in 0.6 M KCl at pH 7, dissociates again on the addition of small amounts of ATP. This dissociation is accompanied by a corresponding drop of viscosity which, at earlier stages of our work, was termed "activity." The drop observed in an actomyosin containing one part of actin to five parts of myosin was called 100% activity. If the myosin showed no drop of viscosity on addition of ATP (activity = 0), it was assumed that the myosin contained no actin.

B. Horváth, repeating experiments of M. Dubuisson,* fractionated myosin with ammonium sulphate. Actin and actomyosin are precipitated at a lower ammonium sulphate saturation than myosin. He thus obtained myosin which on addition of ATP showed an increase in viscosity of 5–20% instead of a drop. If actin-free myosin shows a rise in viscosity on addition of ATP, evidently the "inactive" myosin, obtained previously, might have contained actin, the drop of viscosity on addition of ATP being compensated. In this case our earlier "0 activity" could have been in fact a 20% activity which might have corresponded to an actin content of 3%. If the MW of myosin is 10_0^6 and that of actin 35,000, then 3% actin would mean one actin for every myosin particle, which might have a profound influence on certain reactions of the latter.

* *Experientia*, 2, 413, 1946.

Since these data are rather recent, no final statement can be made. The question must be reinvestigated and earlier results carefully revised.

III. The Fixation of Ions by Myosin

Salt-free myosin is anodic. If KCl is added in increasing concentration, K ions are adsorbed and compensate the negative charge. In the presence of 0.025 M KCl, the myosin is discharged and precipitates. If the KCl concentration is further increased, more K ions are adsorbed and the myosin dissolved again. It was logical to think that the myosin dissolves with a positive charge. Unfortunately the author's laboratory did not have the apparatus necessary for measurement of cataphoretic motion at high salt concentrations.

M. Dubuisson* found myosin dissolved in 0.5 M KCl to be anodic. T. Erdös took measurements of this material in Svedberg's laboratory at Upsala and found crystalline myosin dissolved in 0.5 M KCl also to be anodic with a very weak negative charge;† this necessitated a reinvestigation of the problem of ion fixation, giving special attention to the adsorption of anions.

The work was taken up by W. Sz. Hermann. The main results of one of her experiments are reproduced in Figure 46. (Ordinates are equivalents of K adsorbed by the UW of myosin.) In corroboration of Banga, the figure shows that K is adsorbed according to a curve which, on logarithmic scale, is composed of two straight parts of different gradients. The break, separating "primary" and "secondary" adsorption, corresponds to the maximum of precipitation at 0.025 M KCl. Up to the breaking point, no Cl was adsorbed. At this point, at pH 7.4, three equivalents of K^+ were found to be adsorbed

*Dubuisson, *Experientia* **2**, 258, 1946.

†The first experiments suggest three elementary charges per particle having a MW of the order of 10^6. Possibly this negative charge is of different origin than the original negative charge of the protein, due mainly to its dissociation. Myosin, dissolved in an excess of $CaCl_2$ has a positive charge at neutral reaction. This charge, acquired in strong salt solution, may be an expression of difference of the electro-negativity of the anion and the cation, as suggested by Laki [21] in the case of casein.

per UW as compared with the six K found by Banga at pH 7.5. If the KCl concentration was increased, K was bound still more intensely, as expressed by the steeper gradient. At the same time an equivalent quantity of Cl was bound. The

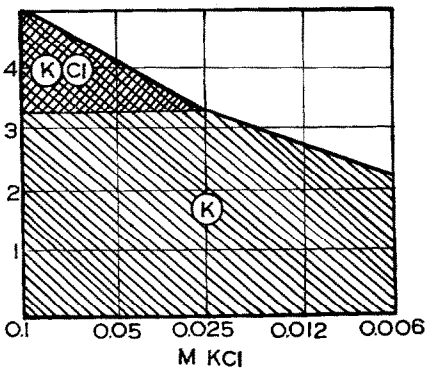

Fig. 46. Fixation of K and Cl ions by myosin in presence of varied concentrations of KCl at pH 7.4. Ordinate: equivalents of K or Cl bound by the UW (17,600 g.) of myosin. In the singly hatched zone only K is bound. In the doubly hatched zone K and Cl are bound in equivalent quantities.

single-hatched area in Figure 46 corresponds to the binding of K^+, the double-hatched area to the binding of equivalent quantities of K^+ and Cl^-.

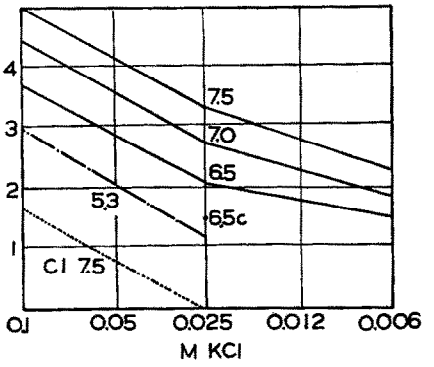

Fig. 47. Fixation of ions by myosin at varied pH. Ordinate: equivalents of ions bound by the UW of myosin. Full lines: fixation of K. Broken line: fixation of K and fixation of Cl. Curve "Cl 7.5" shows fixation of Cl at pH 7.5. The point 6.5 c indicates the quantity of K bound in 0.025 M KCl at pH 6.5 after subtraction of the quantity equivalent to the Cl bound simultaneously.

We may summarize by saying that the negatively charged myosin adsorbs only K ions from KCl. The adsorption of K ions continues until the myosin becomes roughly isoelectric. If the KCl concentration is further increased, the isoelectric myosin binds K^+ and Cl^- ions in equal numbers.

This result is in agreement with the experiment reproduced in Figure 47. The top curve here shows K fixation at pH 7.5. No Cl was found up to the breaking point. From here on, the quantity of K and Cl adsorbed was equivalent (Cl curve, see lower left corner).

The curve 5.3 shows the K fixation of myosin at its IP (pH 5.3). The quantity of adsorbed Cl was equivalent to the adsorbed K throughout, also at 0.025 M KCl.

The curves for pH 7.5 and 5.3 are parallel. The identical gradients indicate that the adsorption of ions is equally intense at either pH. The curve of pH 7.5 is higher, showing that the original negative charge of the protein not only prevents the adsorption of Cl ions, but also promotes that of K ions.

At pH 7 the situation was similar to that in 7.5. At pH 6.5 in the vicinity of the IP, a small quantity of adsorbed Cl appeared at the breaking point, its quantity corresponding to the distance between 6.5 and 6.5c.

The quantity of K^+ adsorbed at the breaking point at different pH's in excess of the adsorbed Cl, corresponds roughly to the number of dissociated COOH groups present, as calculated from the dissociation curve of myosin of Dubuisson* and Dubuisson and Hamoir.†

We may deduce herefrom that myosin is equally capable of binding positive and negative ions and that the fixation of these ions is not due to the dissociation of COOH or NH_2 groups. The dissociation of the COOH group plays a role only as far as the resulting negative charge of the protein prevents the adsorption of anions and promotes that of cations. "Primary adsorption" is the selective adsorption of the cation by the negatively charged protein. "Secondary adsorption" is the adsorption of anions and cations in equivalent quantities by

*M. Dubuisson, *Arch. Internat. Physiol.* **51**, 38, 1943.
†G. Hamoir, *ibid.* **53**, 38, 1943.

the myosin, rendered isoelectric by the adsorbed K^+ or the decrease of pH. The nature of the forces responsible for the binding of ions is one of the most exciting and basic problems of biology.

As has been emphasized, there are reasons to believe that the adsorbed ions retain their charge and the K ions neutralize only the outward charge of myosin. The K and Cl ions, adsorbed in the secondary zone, increase the number of charged points on the protein and promote herewith its hydration and dissolution.

These relations are brought out more clearly by the adsorption of Ca and Cl in the presence of $CaCl_2$ as shown in Figure 48 of W. Sz. Hermann. Up to 0.003 N Ca, no Cl was bound.

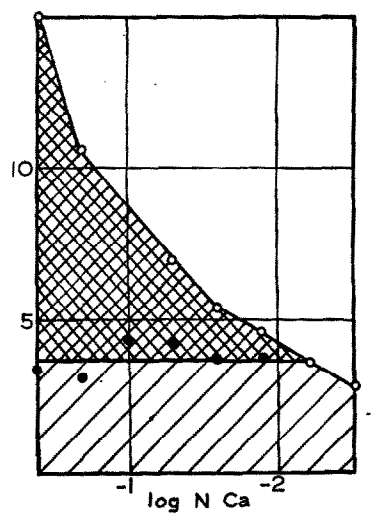

Fig. 48. Fixation of Ca and Cl by myosin in presence of varied concentrations of $CaCl_2$. Ordinate: equivalents of Ca and Cl bound by the UW of myosin. Circles: Ca adsorbed. Points: same after subtraction of a quantity equivalent to the adsorbed Cl. Singly hatched area: Ca adsorption. Doubly hatched area: adsorption of Ca and of the equivalent Cl.

At this $CaCl_2$ concentration the protein has bound four equivalents of metal, thus being approximately isoelectric. From here on, Ca and Cl were bound in equivalent quantities. The upper curve (circles) shows the fixation of Ca, the lower curve (points) shows the same curve after the subtraction of the

quantity equivalent to the adsorbed Cl. The double-hatched area corresponds thus to the adsorption of $CaCl_2$, while the single-hatched area corresponds to that of Ca^{++} without Cl^-.

Figure 49 shows the result of an experiment of Hermann in which KCl was added to myosin in increasing concentration in the presence of 0.001 M $CaCl_2$. The adsorbed Ca, K, and Cl were measured. In the presence of only 0.001 M $CaCl_2$, the myosin was made isoelectric through the selective adsorption of Ca, and was found to bind no Cl. Up to 0.025 M KCl, no K or Cl was bound. At a higher KCl concentration, however, K and Cl were adsorbed by the isoelectric protein in equivalent and increasing quantities. Above 0.1 M KCl, where the K^+ concentration became more than one hundred times greater

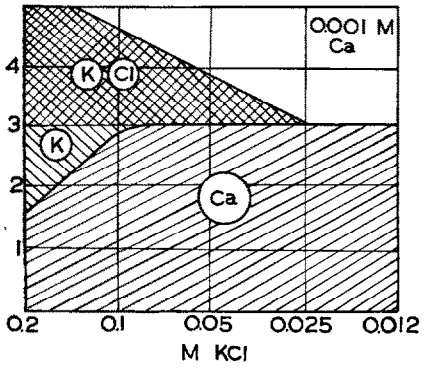

Fig. 49. Adsorption of ions by myosin in presence of 0.001 M $CaCl_2$ and varied concentrations of KCl. Ordinate: equivalents of ions bound by the UW of myosin. "Ca" area: adsorption of Ca. "K" area: adsorption of K. Doubly hatched area: adsorption of equivalent quantities of K and Cl.*

than the Ca^{++} concentration, the K^+ began to expel the Ca from its adsorption.

The question may be raised whether in all these reactions the ions act merely as electrically charged balls, distinguished only by the number of charges and ionic radius, or does their further individuality, their finer structure also come into play. Although physicists may vigorously object to this latter

*In a number of later experiments the adsorption of Ca began to decrease in the presence of 0.025 M KCl to become asymptotic with one Ca adsorbed.

possibility, the observations support it, the ions having a specific influence on the protein which can hardly be explained either by the ionic radius or the number of charges.

Mg, for instance, greatly enhances contraction of actomyosin in the presence of KCl and ATP. G. Rózsa compared the effect of Mg with that of Be, Ca, Sr, and Ba, and found it rather specific. Cu completely changes the properties of myosin: it makes the protein very elastic and unable to contract. If the Cu is washed out with cyanide, original physical properties return [Dubuisson] and the protein becomes contractile again.* Ca myosinate is not contractile, in contrast with Mg myosinate. If part of the Ca is replaced by Mg, the myosin regains contractility. Ca myosinate is very active as ATP-ase; Mg myosinate is completely inactive. In the presence of actin, both are equally active.

Actin offers still more striking examples of the specific activity of ions (e.g., K^+ and Na^+).

IV. Enzymatic Activity and Contractility of Myosin. Protins

The main achievement reported in the first part of this book was that old "myosin" could be pulled to pieces, into actin and myosin proper. Lately we succeeded in pulling myosin to pieces, in spite of the fact that it can readily be crystallized and behaves on recrystallization as a homogeneous substance. Since the foregoing parts of this book were written, my laboratory was led to regard myosin as a rather involved system in which a number of protein-like substances are attached to a skeletal substance. The skeleton as well as the adsorbed proteins are, in themselves, inactive, their system being capable only of enzymatic reactions or contraction. Even lipids seem to be an integral part of this system.

The adsorbed proteins all seem to belong to the same remarkable group of substances, characterized in impure condition by their stability in 0.1 N HCl at 100° (15 minutes). They can be treated without loss with N HCl or precipitated

*G. Rózsa, unpublished.

with trichloracetic acid. These substances will be called "protins" (prosthetic-proteins) to distinguish them from other proteins. H. M. Kalckar was the first to describe in muscle an acid- and heat-stable catalytic protein, his "myokinase."

This problem was pursued along two different lines. F. Guba studied contractility of actomyosin. He prepared protin-free actomyosin. Such actomyosin does not contract with ATP and ADP but can be reactivated with watery muscle extract. I. Banga studied the enzymatic activity of myosin, namely dephosphorylation and deamination of ATP, and dephosphorylation and deamination of ADP. Both workers agree that the protins, involved in the reaction with ADP ("ADP-protins") are much more easily removed from myosin than the protins involved in reactions with ATP ("ATP-protins"). Accordingly, they succeeded much sooner in detaching the ADP-protins from the skeletal substance and reactivating this latter by watery extracts of the muscle: protein II of Banga and the chromoproteid of Guba, described in the first part of the book, are what we now call ADP-protins.

The protins involved in the reaction with ATP are detached from the myosin skeleton, but under specific conditions. Actin, which renders removal of ADP-protins difficult, makes the removal of ATP-protins impossible; the myosin must thus be entirely free of it. The dissociation of myosin into skeletal substance and protins is promoted by acid reaction. The skeletal substance, however, is very labile and denaturates readily under the influence of H ions. Thus one is rather limited in the pH range, the more so because the dissociation of the complex is a rather slow reaction and demands protracted treatment. There is but a narrow margin between the range of denaturation and dissociation. Protins, adsorbed to the skeletal substance, greatly increase the solubility of the latter. Myosin, rich in protins, is clearly soluble in 0.5 M KCl, becoming more insoluble and cloudy as the protins are removed. The solubility of the skeletal substance can be increased again by the re-adsorption of protins, but if the protins are completely removed, changes become irreversible. So Banga was able to reactivate the ATP-ase activity of her myosin only if this was not reduced by more than 85%.

The results of the contraction test and the phosphatase test are not yet sufficiently co-ordinated. In one case the myosin of Guba, which gave no more contraction, still showed 50% ATP-ase activity. It seems probable that both protins, the one involved in contraction ("ATP-c-protin") and the one involved in dephosphorylation are identical, but contractility disappears if the protin concentration drops below a critical value, which corresponds to 50% inactivation of the phosphatase activity.

These results are very recent and do not allow more quantitative statements at this time. This is true especially for the protin involved in deamination of ATP ("ATP-N-protin"). This reaction has been discovered lately by Banga, whose results allow only the statement that this protin is different from that involved in dephosphorylation of ATP or in deamination of ADP.

Singher and Meister* reported the preparation of myosin, inactive as phosphatase. It is difficult to say whether the inactivity was due to the removal of protins since myosin can be inactivated in many ways. Their method being similar to ours, it seems likely that they removed protins, provided their myosin was free of actin.

Myosin can also be inactivated for the ADP contraction test by the removal of a lipid, which has been obtained in crystals and seems to be a cerebroside. Its addition to the myosin reactivates the latter. Myosin can also be inactivated by storage at alkaline reaction in contact with air [Guba]. Such a myosin contracts no more but can be reactivated by cysteine. Inactivation, in this case, is due probably to the oxidation of SH groups. Bailey and Perry† found SH-groups essential for the actin-myosin link, while Singer and Barron‡ found SH groups involved in phosphatase activity. Naturally, it is important to distinguish between autoxidative inactivation of protins and of the skeletal substance.

The quantities of actin, which may make ATP-protin

J. Biol. Chem. **159**, 491, 1945.

†K. Bailey and S. V. Perry, *Proc. Biochem. Soc.*, 24th meeting, 1946.

‡ *Proc. Soc. Exp. Biol. Med.* **56**, 120, 1944, quoted after Bailey and Perry.

inseparable from the myosin skeleton, are too small (3%) to explain this effect in a rough physical way by supposing that the protin is entrapped between the myosin and actin particles. We must suppose that the actin increases the affinity of myosin to protin.*

V. ATP-ase Activity of Muscle at 0°C

A. Biro and A. E. Szent-Györgyi, measuring the ATP-ase activity of muscle and myosin at varied temperature, have found an exceedingly low temperature quotient. The increase in rate pro 10° was but 30% instead of the 250–300% usually observed in other reactions. This suggests that the enzymatic activity has a rather involved mechanism and is limited by a reaction with a low quotient. The relatively low activity of myosin as an enzyme is also suggestive of such an involved mechanism. Other systems in the realm of physiology make such a mechanism rather probable. The function of classical enzymes like trypsin or pepsin is to decompose their substrate as rapidly as possible. The decomposition of ATP would have no sense if it would not fit into a more complex pattern of reactions which together form the contraction cycle.

But whatever the reason for such a low temperature quotient may be, it opens valuable experimental possibilities for studying the ATP-ase action of muscle at 0° where there is no contraction. So the enzymatic activity can be studied without being complicated by such gross physical changes as contraction. Once we have determined what uncontracted muscle does, it will be easier to find out what is due to contraction itself. If both the physical state and the rate of the enzymatic reaction would change with temperature, the two variables would make interpretation of results rather difficult.

Biro and Szent-Györgyi studied first the ATP-ase activity of freshly minced muscle, no ATP being added. Such a muscle

*This effect may have its physiological bearing. It is not unlikely that the influence is mutual and that changes in the protin (e.g., its phosphorification) after the affinity of myosin to actin, inducing association or dissociation. The latest results of Guba suggest that protins are involved in actomyosin formation.

contains ATP in high concentration. They found that at a lower KCl concentration the muscle splits its own ATP rapidly; at a higher concentration it does not. The borderline lies somewhat below 0.16 M. The transition is quite critical: at 0.15 M, splitting is maximal; at 0.16 M KCl, there is almost none. The same was found to be true for the two instances when ATP was added from without to the suspended and washed muscle.

A solution of 0.16 M KCl is roughly isotonic with Ringer, serum, or resting muscle. Just below isotony there lies thus a critical ionic concentration at which muscle begins to split its own ATP, or ATP added from without. Resting muscle is just above this limit.

It will be remembered that the same ionic concentration, between 0.15 and 0.16 M KCl, was found to be critical also for contraction, as shown by the lower half of Figure 40. Below this concentration the ATP, present in resting muscle, induces contraction; above this concentration it does not. The ionic balance of resting muscle is such that the muscle does not contract and does not split its ATP, being just beyond the critical level below which it splits and contracts.

If ATP is added to washed or unwashed muscle at 37°, the muscle contracts up to 0.45 M KCl. The first experiments of Biro and Szent-Györgyi show that it also splits ATP. If any conclusion may be drawn from these first results, one would have to conclude that it is contraction which enables the muscle to split ATP above the critical level.

VI. Energetics

In the first part of this book, experiments of L. Varga were described which permitted calculations of the heat- and free-energy change in muscular contraction. The numeric values obtained related to the molar quantity of the contractile matter, but we did not know what this molar quantity was. The molar quantity of extracted myosin is in the range of 10^6 g. It has been shown, however, that the myosin particles, in muscle, are associated with higher structures. Moreover the

contractile substance is not myosin but actomyosin, containing in muscle 1 g. of actin to 2.5 g. of myosin. If the relation of the MW of actin and myosin is 70,000:1,000,000, this means that about six actin particles unite with one myosin particle. The situation is complicated further by the association of the actin particles; thus we are at a loss as to what to call the molar quantity.

To approach this problem, Varga repeated his muscle experiments on actomyosin threads. The curves obtained were identical with those obtained on muscle, which shows that the association of myosin particles in muscle to higher structures does not change the molar quantity, which is the same in muscle and in extracted myosin, where the myosin system is disintegrated to particles of MW of the order of 10^6.

The next question to be answered was, to what extent did actin comprise the molar quantity of contractile matter. For this reason actomyosins of different relative actin and myosin content were prepared. As shown previously by Erdös [33] an actomyosin containing 8% of actin is as contractile as an actomyosin containing 30%. The experiment showed that the variation of the actin content did not appreciably alter the curves obtained. The energy change of the system is governed thus by changes within the myosin particle, the molar quantity of which is in the range of 10^6 g.

It is generally accepted that the energy of the acid anhydride link of the pyrophosphate group of ATP is used in muscular contraction. The free-energy change of the splitting of this link is unknown; its total energy change is 11,000 cal. If the free-energy change and the change of total energy are not very different, then the splitting of one link suffices to supply the energy for the relaxation of one myosin particle which, as shown by Varga, is 7,000 cal. (37°).

Professor Lundsgaard was good enough to inform me that a muscle is capable of seventy contractions in iodoacetic acid poisoning when the regeneration of the ATP is inhibited. If the MW of myosin is 10^6, there are about seventy molecules of ATP in resting muscle for every molecule of myosin. If muscle contains 10% myosin,* then 10,000,000 g. of muscle contains a

*Banga has recently found 10% myosin in muscle on exhaustive extraction.

molar quantity of it. If the free-energy change of contraction is 7,000 cal., then in a single twitch 1 g. of muscle should be able to lift about 30 g. a distance of 1 cm., which is in fair agreement with physiological experience.

VII. The Continuum Theory

J. Boros obtained the photoelectric effect in gelatin phosphors by spectrally decomposed light. The spectral distribution of this effect has not been measured yet quantitatively, but the first observations suggest that its maximum coincides with the maximum of the light adsorption of the dye. There has been found a linear relationship between the logarithm of the concentration of the dye in the gelatin phosphor and the photoelectric effect. These results do not constitute any progress in the theory, but both results are rather reassuring that the photoelectric effect, observed on illumination, was not due to thermal changes.

J. Gergely made a few observations on the phosphorescence of myosin solutions in the presence of rhodulin-orange. Myosin was employed in a 4-mm.-thick layer. The KCl concentration was varied between 0 and 2 M and the pH from 7 to 9. At the concentration used (1–2%) all solutions showed DRF and phosphorescence. On dilution, phosphorescence always disappeared before the DRF.

If the myosin dissolved in 0.5 M KCl was diluted until it gave no more phosphorescence (0.5–1%) and then an actin solution was added which in itself showed no phosphorescence (one part of actin for five parts of myosin) the DRF and the phosphorescence appeared again. If the actomyosin was brought to dissociation by the addition of ATP or by the raising of the salt concentration above 1 M, the phosphorescence disappeared. This shows that actin and myosin, when uniting to form actomyosin, pool their valency electrons into one common system. Actomyosin is thus not simply actin plus myosin; it is a new substance.

The phosphorescence of muscle (dyed with rhodulin-orange) was not altered by contraction or heat denaturation.

The experience collected with actin, myosin, and protins cannot be taken as direct evidence for the correctness of the continuum theory. Without this theory, however, it would be difficult to explain how these proteins, when uniting to a complex, develop entirely new qualities, as enzymatic activity or contractility, and how a small actin particle, attached to a relatively huge myosin particle, should be able to change the affinity of this latter toward a protin.

VIII. Conditions in Muscle

Since it has been shown that myosin adsorbs in its secondary zone cations and anions in equivalent amounts, the question may be raised which is the anion in muscle to balance the K^+. There can be little doubt that the primary zone is occupied by bivalent ions, chiefly if not wholly by Mg. According to the curves of Hermann, at the K- and Na- ion concentration of muscle we can expect to find three alkali metal ions bound per UW in the secondary zone.

In the presence of KCl, myosin adsorbs K and Cl in its secondary zone. Muscle, however, contains but very little Cl; most of the anions are organic, chiefly PO_4 bound to organic radicles.

Muscle, under normal conditions, contains about 0.005 M ATP, the greatest part of which is bound to myosin. This is equal to one ATP bound per UW of myosin, which would be just sufficient to balance the three K ions adsorbed, ATP having three negative charges at neutral reaction. It is true that Banga and Hermann have found evidence indicating that the negative charge of the adsorbed ATP is balanced by K ions of the surrounding solution, but I am inclined to believe that these results do not reflect conditions *in vivo* and are due to specific conditions prevailing during precipitation.

Assuming thus that the anion adsorbed by myosin in the secondary zone is actually ATP, a number of observations become intelligible.

There are three critical concentrations of KCl in regard to

ATP adsorption: 0, 0.1, and 0.16 M. At concentration 0, there is practically no ATP adsorbed to myosin, even if the primary zone is occupied and the negative charge of myosin compensated by bivalent ions. If there is no free positive charge on myosin, it is unable to bind any ATP. At 0.1 M KCl, in most experiments, the adsorbed quantity of ATP reaches maximum. At this KCl concentration there are two K ions bound in the secondary zone. This suggests that myosin is capable of binding the maximum amount of ATP when it has at least two alkali metal ions adsorbed to balance two out of three negative charges of ATP (at neutral reaction ATP has three negative charges). At the third critical concentration, lying somewhat below 0.16 M KCl, the adsorbed ATP anions seem to be bound most strongly. At this concentration three K ions are adsorbed to balance completely the three negative charges of ATP. Now the ATP seems to be bound very strongly and to be unable to react with the ATP-protins adsorbed. If the KCl concentration falls below this critical level, the number of K ions adsorbed falls below three and the ATP is no more fully balanced, its adsorptive power is decreased; it interacts with the protin, is split, and induces those changes which eventually lead to contraction.

This may also explain the difference in the action of ATP present in muscle and the ATP added from without. It has been shown in the second part of this book that above 0.16 M KCl the relatively great quantities of ATP present in muscle are unable to induce contraction while minimal amounts of ATP, added from without, do so, though there is no reason to suppose that there is any difference in chemical structure. According to the above experiences, the adsorbed ATP may be unable to interact with the protin, while the free ATP is free to do so.

At last the question may be raised concerning the nature of the interaction of protin and ATP. The simplest and most probable assumption is that there is a transphosphorylation, the "myosin" being phosphorylated. This phosphorification of "myosin" could induce the rearrangement of charges which leads eventually to contraction, the whole system losing 7,000

cal. of free energy. If the contracted system would now detach its acquired phosphate, it could return to its original state, taking the 7,000 cal. necessary from the splitting of the pyrophosphate link. If the free energy of this latter is 11,000 cal., relaxation also would go hand in hand with a fall of free energy by 4,000 cal. Thus both contraction and relaxation would be spontaneous processes accompanied by a loss in free energy, and the splitting of ATP would seem logical. It is not even necessary to suppose a transphosphorification to be able to accept this picture. An adsorption of the ATP to the protin may do the same, having been shown that substances, adsorbed to proteins, may take part with their electrons in the structure of the whole; thus it is possible that a mere adsorption of the ATP to the protin may induce the rearrangement of electronic distribution and allow the energy of the pyrophosphate link to be communicated to the whole system.

Naturally, until we know more about conditions in resting muscle we must not adhere too strongly to the details of any such picture. It is still possible that actomyosin, in resting muscle, is in the dissociated condition and that phosphorification of the protin (or the adsorption of the ATP to protin) only increases the affinity of myosin to actin, causing association to actomyosin which, in its turn, might entail the intramolecular rearrangement of charges leading eventually to contraction. If this electric disturbance (or the consecutive release of water) disturbs the K adsorption in the neighboring micels, thereby causing release of ATP, the change becomes self-propagating.

If muscle is stored for a few hours at room temperature, it loses its excitability. G. Rozsa found that excitability is lost when the ATP concentration of muscle falls below ½ of its original level. It is possible that in this case the predominating K^+ adsorption prevents the release of the adsorbed ATP.

IX. The Nature of Cross-Striation

M. Gerendás and G. Matoltsy have tried to repeat the author's experiment in which insect muscle fibers were rotated

under the microscope. Under these conditions, the author has observed a shift of the cross-striation and concluded therefrom that cross-striation must be an optical phenomenon and cannot be due to a segmental differentiation of the fiber.

Gerendás and Matoltsy, working under better conditions, were able to register the results photographically. They showed that under the conditions of the author's experiments a continuous shift of cross-striation can actually be observed on rotation but that this shift is an optical phenomenon only and there is no real shift of striations. The explanation is this: since the fibers never lie perfectly axially, rotation shifts them out of focus. To facilitate observation during rotation, the author did not focus his microscope on the equator of the fiber but as far from the equator as visibility permitted, say above the equator. The fiber was rotated and moved upwards through the focus as far as possible. Gerandás and Matoltsy showed that if we focus the microscope in this manner above the equator and then move it below the equator, there is always a shift of the cross-striation by half a period. If the above manipulation is repeated, one gets the impression of a continuous motion. This, however, is but an optical phenomenon and there is no real shift of cross-striation, which can be shown if we use objective methods of registration, focus consistently in the same plane, and use as fixed point some point in our preparation outside the fiber. The complete series of pictures obtained on a full rotation, if put together, reveals that no shift has occurred.

To obtain further information about the nature of cross-striation, Gerendás and Matoltsy undertook the microscopic examination of mammalian cross-striated muscle, the myosin of which was removed by thorough extraction with Weber's salt solution containing ATP. In plain light, cross-striation was found unchanged, but the DR had greatly diminished or had disappeared completely.

They now treated the extracted muscle with strong (0.6 M) KI, which depolymerizes and dissolves the actin. The result was unexpected: the cross-striation was still present but the positive DR of the muscle turned into a negative one and the

former I-band became (negatively) double-refracting, while the former Q-bands became isotropic. The negative double refraction of the I-bands was equal (4.10^{-4}) to the positive DR of the Q-bands of untreated fibers.

These observations admit a very simple explanation: the I-bands contain a negative double-breaking substance, which compensates the positive DR of the continuous, positively double-breaking contractile actomyosin filaments. This substance cannot be identical with Bailey's tropomyosin, which has a positive DR. The negative double-breaking substance can be dissolved with urea salt solution and displays, after alkalization with NaOH, a negative DRF.

This protein has been described previously by I. Banga and myself [2]. We showed that the structure of all animal tissues examined is built of fibrous proteins which can be dissolved partly in urea salt solution and partly in alkaline urea salt solution.* It was also shown that muscle contains such a protein which can be detected after the extraction of myosin. Heart muscle was found to be especially rich in this protein.

It is hoped that these findings will open the way to the understanding of the function of the muscle fiber as a whole. At the same time they open the question again, whether cross-striation corresponds to a segmental differentiation, or whether it is the result of the periodically changing optical properties of a continuous structure. Possibly the negative double-breaking substance of the I-bands fills the space between the actomyosin filaments continuously, but its particles have a periodically changing orientation, as the particles of the fibrous colloid in van Itterson's capillaries.

*The urea salt solution had the following composition: 0.6 M KCl, 0.01 M Na_2CO_3, and 0.04 M $NaHCO_3$ and 30% urea; thus it corresponded to Weber fluid + urea. The alkaline urea salt solution had the same composition but contained 2% NaOH in addition.